Comparing density of lumpfish (*Cyclopterus lumpus*) used in aquaculture to different welfare indicators

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1 Abstract

Farmed lumpfish are commonly used as cleaner fish in the salmonid aquaculture industry, but a knowledge gap exists with regards to their density. Filling this knowledge gap is of importance, as the lumpfish have no swim bladder and thus relies heavily on its lipid storage for buoyancy, i.e. the density difference between the fish and its surroundings. The aims of this study were to measure the density of lumpfish and investigate the correlation between density and different welfare indicators. Furthermore, the results were compared to existing literature that investigated density of adult wild lumpfish.

138 lumpfish were sampled at four different aquaculture sites situated in the Faroe Islands. The lumpfish were caught with a dipnet and euthanized with Finquel. Weight in water and air was measured and the density was calculated. Welfare indicators such as Fulton´s K, liver colour, hepatosomatic index, length, weight, skin, fin, and stomach fulness scores were measured.

The average density of the juvenile lumpfish was 1.030 g mL^{-1} which was reminiscent of existing literature. Fulton´s K, stomach score, and length had a negative influence on density, while the hepatosomatic index had a positive influence on density. Tukey's post hoc test showed that liver colour also influenced density, but the groupings were too broad to conclude anything.

The knowledge gained from this study will help the industry improve their understanding of the welfare parameters used for lumpfish. Additionally, the knowledge might also help the aquaculture industry improve their husbandry and feeding practices.

Keywords: aquaculture, buoyancy, cleaner fish, *Cyclopterus lumpus*, density, lumpfish, welfare indicators

2 Acknowledgements

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Contents

3 Introduction

Since its inception, the Atlantic salmon aquaculture industry has had problems with a marine parasite called the salmon louse *Lepeophtheirus salmonis* (Costello, 2009; Torrissen et al., 2013; Fiskehelserapporten, 2020). The salmon louse is an ectoparasite, that attaches to the skin of the salmon and feeds on mucus, blood, and skin (Pike & Wadsworth, 1999; Treasurer, 2018). High lice infestation numbers can cause physical damage to the skin which can lead to osmoregulatory failure, secondary infections, immunosuppression and chronic stress (Grimnes & Jakobsen, 1996; Nolan et al., 1999).

The damage caused by *L. salmonis* has shown to be a limiting factor in industry growth and it has been estimated that it is the greatest cause of financial loss in the salmonid aquaculture industry (Costello, 2009). The loss of production caused *L. salmonis* in Norway alone was estimated to be 525 million USD in 2019 (Jensen et al., 2020). Therefore, it is of upmost importance for the industry to identify effective delousing treatments.

3.1 Different delousing methods

The aquaculture industry is actively fighting the lice with different delousing treatments that could be categorized into three groups: chemical, mechanical, and biological treatments (Barrett et al., 2020). Several methods of louse prevention have also been developed, including louse nets, functional feeds, vaccines, land-based aquaculture, offshore aquaculture, closed pens and integrated multi-tropic aquaculture (filter feeders) (Bartsch et al., 2013; Barret et al., 2020).

The chemical treatments consist of use of primarily five chemicals (Avermectins, benzoyl ureas, disinfectants, organophosphates (OPs), and pyrethroids (PYRs)), that interact with the lice in different ways. The chemicals are either introduced to the fish through feed or with a bath treatment. The disadvantage of using chemicals as a delousing method is that the sea louse is developing resistance to them. The industry temporarily solved the issue by rotating the usage of the drugs. However, the overall louse resistance to the drugs has since then increased, making this solution inefficient (Aaen et al., 2015; Samuelsen et al., 2019; Fiskehelserapporten, 2020).

The mechanical treatments consist of different mechanical treatments such as hydrolicing (hosing the salmon) and thermolicing (exposing the salmon to hot water). The mortality rate from mechanical delousing is high, up to 31% for thermolicing and 25% for hydrolicing (Overton et al., 2019). However, this was the most common type of delousing method in 2017.

The biological delousing treatments consists of letting different fish species such as Ballan wrasse (*Labrus bergylta*) and lumpfish (*Cyclopterus lumpus*) (Haugland et al., 2020) which exhibits cleaning behaviour, coexist with the salmon in the fish pen (Powell et al., 2018a). The newest addition of species to be used in this cleaning method is lumpfish, which have shown cleaning behaviour in colder regions, where Ballan wrasse would not be effective (Imsland, et al., 2014a; Yuen et al., 2019). Additionally, lumpfish is the only existing alternative in the Faroe Islands as the Ballan wrasse is not a native species. Lumpfish have a very high specific growth rate (1.5-3% day^{-1}) (Nytrø et al., 2014). The rearing cycle is also easier to complete compared to Ballan wrasse (Imsland et al., 2018). These characteristics make them very attractive to the industry. The lumpfish is also a more sustainable alternative to the Ballan wrasse as the lumpfish are reared while Ballan wrasse is mainly supplied from wild fisheries (Norwegian Directory of fisheries, 2019). The lumpfish were introduced to the industry in 2012 (Fig. 3.1) and have since then become the most popular choice of cleaner fish species, (Imsland et al., 2014a-c, 2015a-b, 2016a-b; Haugland et al., 2020). Although the lumpfish have become the most popular choice of species for cleaner fish use, the general knowledge about the species is lacking. Powell et al. (2018b) lists several gaps in knowledge, such as proper feed composition for different life stages and research on health and stress management.

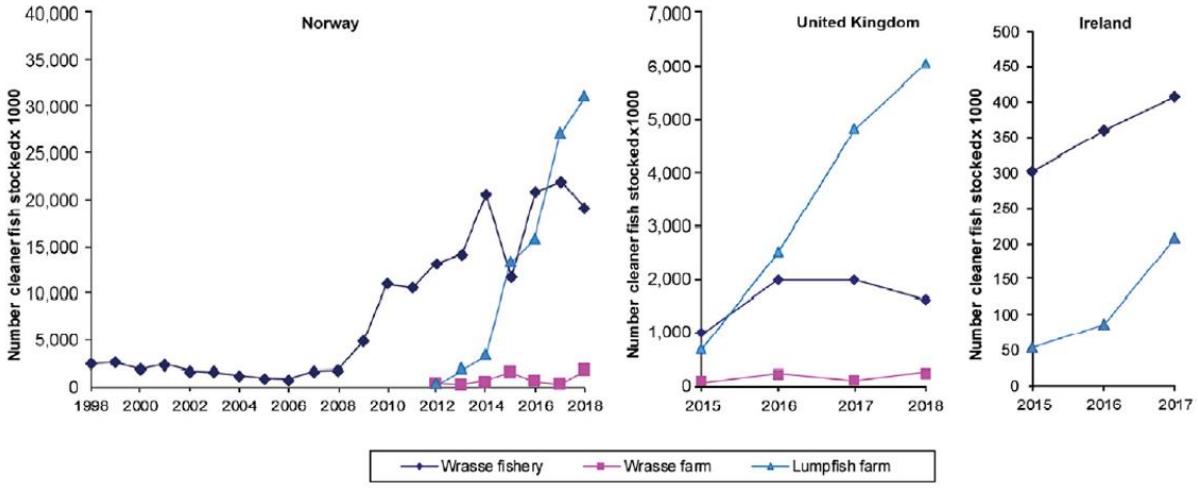

Figure 3.1. Overview of produced lumpfish and Ballan wrasse in Norway, United Kingdom and in Ireland. Modified from Haugland et al. (2020).

3.2 Lumpfish

The lumpfish are a bony fish (class: Osteichthyes, infraclass: Teleostei), belonging to the Order Scorpaeniformes (Order might be Perciformes according to Betancur-R et al. (2013)), family Cyclopteridae and are the only species of the genus Cyclopterus (Powell et al., 2018a). The lumpfish are globiform and have no swim bladder, instead they rely heavily on a low density body for buoyancy (Davenport & Kjørsvik 1986). Also, the lumpfish have a characteristic abdominal sucker, formed from the pelvic fins (Davenport & Kjørsvik, 1986), that they use to latch on surfaces when they are resting. umpfish distributed in the boreal region of the east and west coasts of the North Atlantic, but have also been found as far south as Spain and Portugal, and as north as Svalbard and John Mayen (Powell et al., 2018a). Lumpfish can be found in shallow waters during breeding season (Davenport & Kjørsvik, 1986). However, lumpfish have also been found as deep as 868m (Powell et al., 2018a). Young lumpfish feed on a wide variety of food items, such as copepods and different species living in seaweed such as amphipods and crustaceans (Daborn & Gregory, 1983; Imsland et al., 2014c, 2015a; Haugland et al., 2020).

Lumpfish used as cleaner fish have also been observed to feed on available prey, however it has been shown by Imsland et al. (2015a) that these lumpfish are opportunistic feeders and

thus feed on the most abundant feed source, which most often was salmon feed. However the abundance of different feed types is dependent on seasonality as during summer months lumpfish pray more on zooplankton and fouling organisms on the net pens (Eliasen et al., 2018). Lumpfish also have their own feed that is developed for their specific need, and different feeding mechanisms have been tested, such as the use of feeding blocks instead of pellets (Imsland et al., 2019).

3.3 Welfare indicators

Welfare indicators (WI) are observations or measurements that provide information about whether the animals welfare needs are met. WI are used in research on both wild animals and lab animals, but in the industries, operational welfare indicators (OWIs) are preferred. OWIs are indicators that can be realistically used in industrial areas such as at a fish farm. Examples of operational welfare indicators being used for lumpfish can be seen in Table 3.1.

Table 3.1. Different OWIs used in three different published articles.

Imsland et al. (2020)	Eliasen et al. (2020)	Gutierrez Rabadan et al. (2021)
Body condition score (0-3)	Tail fin score (1-3)	External body damage score (0-1)
Tail fin score (0-3)	Skin score (1-3)	Fin damage Score (5-point Likert)
Other fins score (0-3)	Eye score (1-3)	Eye condition score (0-2)
Deformities score (0-3)	Stomach content (1-6)	Eye darkening score (5-point Likert)
Cataract score (0-3)	Liver colour (1-6)	Suction cup deformity (5-point Likert)
Eye ulceration score (0-3)	Weight length relationship	Relative weight (Wr)
Condition factor score (0-3)		

Most of these indicators are very easy to score and they give an indication on the overall health of the lumpfish. Some of the indicators used in this study can be seen in Fig. 3.2. Indicators such as eye damage, fin damage and cataract are used on other species too, however it is important to note that different species might react differently to damage, thus the severity of the indicators must be adapted to each species (Noble et al., 2018).

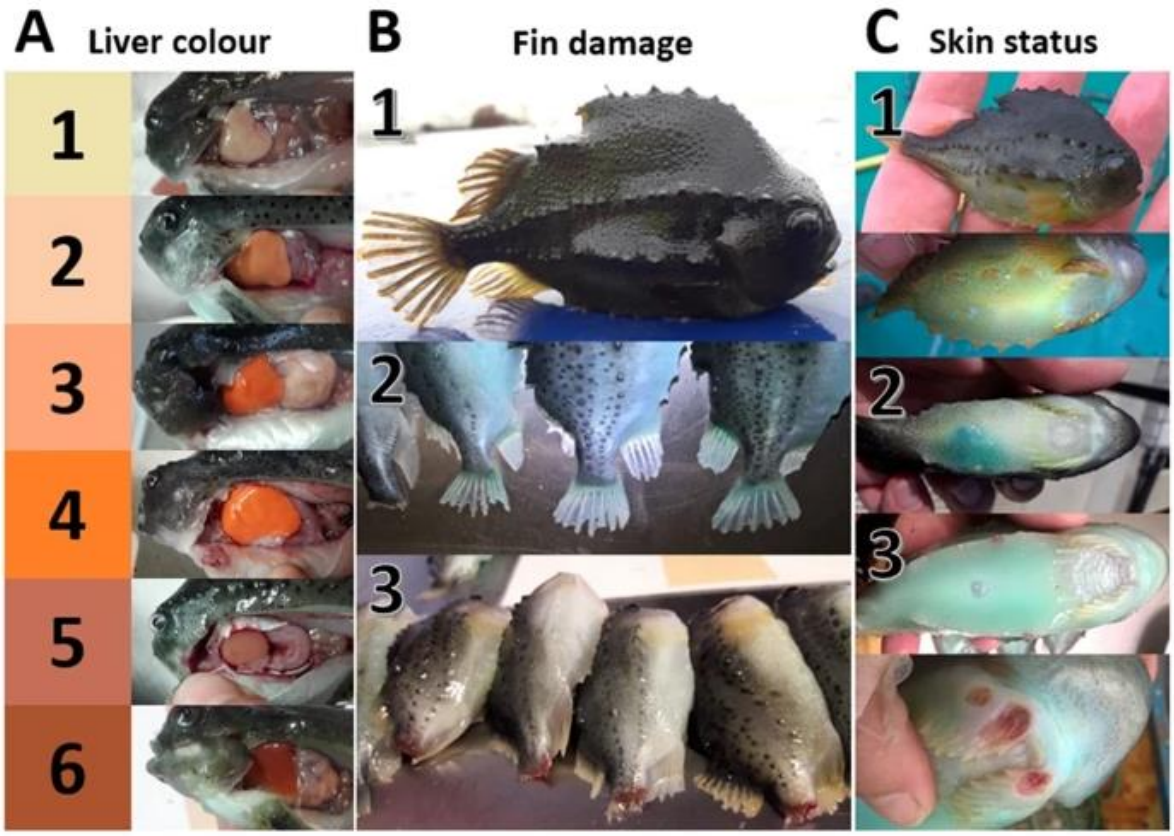

Figure 3.2. Three of the OWIs that were used in this thesis. (A) Liver colour score. (B) Fin score. (C) Skin score. The figure is from Eliasen et al. (2020).

The welfare of the lumpfish in the net pens is questionable as of now, because of the high mortality rates in the net pens, which on average is 48% and varies from 39% to 100% (Imsland et al., 2020; Rådet for dyreetikk, 2020). To be able to assess the health of lumpfish in the sea pens, thus reducing mortality rates, OWIs need to be used. In the Faroe Islands, routine monitoring of lumpfish includes looking at several OWIs such as, fin damage, skin damage, suction cup damage, eye damage and liver colour (Eliasen et al., 2020). Indicators such as skin damage and fin damage directly point towards physical damage on the fish and thus on the overall health of the fish. Eliasen et al. (2020) has suggested that the liver colour might be used as an indirect measurement of welfare. A pale liver might indicate disease, but only if the lumpfish are larger than 50 g and previous samples, from routine monitoring, have had orange livers before, but even then, it is uncertain. An orange liver indicates a high carotenoid reserve and thus an healthy lumpfish, while a dark liver indicates lowered lipid reserves which indicates that the lumpfish are starving (Eliasen et al., 2020). In addition to the

OWIs used in Table 3.1, indices have also been made, that make it possible to interpret the indicators in a way that makes it possible to grade them all together. Such a grading system is used to determine whether any action is required to increase the welfare and what those actions might be. In Table 3.2 an example can be seen of the grading system that was developed by Imsland et al. (2020).

Table 3.2. Mean welfare score that was made from operational welfare indicators*

Mean welfare score	Evaluation	Action required
0-11	No to minimal deterioration.	No action required.
	Health status satisfactory.	
From 11 to 16	Higher incidence of compromised health.	Implement improvement measure if applicable.
	Health status deteriorating.	Cease handling/sampling tasks.
		Consider further assessment.
Over 16	Evidence of further extensive health deterioration.	Immediate action plan required.
	Health potentially compromised.	Consultation with approved veterinary.

*The Table is modified from Imsland et al. (2020)

3.4 Lumpfish buoyancy

Buoyancy is the force that counter affects gravity of an object that is floating in a liquid, the force itself comes from the liquid that is exerted by the object (Young et al., 2011). Buoyancy is highly relevant for aquatic species, as it is one of the main forces that keeps them from sinking (Stevens, 2011; Macaulay et al., 2020), others being hydrodynamic force produced from swimming activity (Pinte et al., 2019). It Is possible to measure buoyancy directly but a more common and easy method of looking at buoyancy is using density. Density is defined as mass per unit volume (Young et al., 2019) and can be defined as the following equation:

Equation 3.1

$$\rho = \frac{m}{V}$$

Where:

$\rho = Density$

$m = Mass$

$V = Volume$

The only previous study that looks at buoyancy of lumpfish is the study of Davenport & Kjørsvik (1986). However, buoyancy in other fish species has been investigated. Pinte et al. (2019) measured the buoyancy of the liver in deep sea sharks and the buoyancy function of different lipids where examined in Phleger (1998). Phleger (1998) found that the major lipids, that have a direct role in buoyancy were wax esters, squalene, and alkyl diacylglycerols. Squalene and alkyl diacylglycerols were found in the liver of marine fish, but to the authors knowledge, no one has looked at these lipids in lumpfish. The average liver density in the study of Davenport & Kjørsvik (1986) was higher than the average density of the entire lumpfish used in that study. This indicates that the liver negatively affects the buoyancy of the lumpfish. What was found to increase buoyancy of the lumpfish, was the subcutaneous jelly, muscles, blood, and different fluids. However, the fish that Davenport & Kjørsvik (1986) investigated weighed between 1.3 kg to 2.6 kg, which is much larger than the average weight of a lumpfish used for biological delousing in an aquaculture sea pen (< 300 g, Imsland et al., 2016b, 2021). Thus, the density, and its possible link to the welfare status, of the lumpfish that are used in the aquaculture industry should be examined further.

3.5 Aim of study and research hypotheses

Farmed lumpfish are widely used as cleaner fish in the salmonid aquaculture industry in Norway, Scotland and the Faroe Islands. Because lumpfish have no swim bladder, they rely on their lipid storage for boyancy, but little is known about the bouyancy of lumpfish. In the Faroe Islands, routine monitoring of lumpfish has shown large variations in their condition (Fulton´s K), as well as in their liver colour. As the liver is a lipid storage for fish and Fulton's K reflects the fish condition, the density of the fish might correlate with these two indicators. Additionally, the density of the lumpfish might vary with other welfare indicators.

The aim of the study was to fill the knowledge gap that exists in the literature about the density of lumpfish, used in aquaculture, and compare the density with different welfare indicators. The study was devided into four research questions that each compare density to different welfare parameters.

Research Hypotheses:

The lumpfish density is influenced by its condition (Fulton´s K) and welfare status (liver colour, hepatosomatic index, fin score, skin score, stomach fulness score, and length/weight). The H_0 and H_1 hypothesis for each research question is shown below.

To validate the research hypotheses the following research questions were investigated:

1. Is lumpfish density influenced by condition (Fulton´s K)?
2. Is lumpfish density influenced by welfare status (liver colour and general health)?
3. Is lumpfish density influenced by hepatosomatic index (liver weight as a percentage of the whole-body weight)?
4. Is lumpfish density size related (length and weight)?

Research question 1

H_{01} = There is no relationship between density and Fulton´s K

H_{11}= There is a relationship between density and Fulton´s K

Research question 2

H_{02} = There is no relationship between density and the different welfare variables

H_{12}= There is a relationship between density and the different welfare variables

Research question 3

H_{03} = There is no relationship between density and Hepatosomatic index

H_{13}= There is a relationship between density and Hepatosomatic index

Research question 4

H_{04} = There is no relationship between density and weight

H_{14}= There is a relationship between density and weight

Research question 5

H_{05} = There is no relationship between density and length

H_{15}= There is a relationship between density and length.

4 Material and Methods

4.1 Study species and location of study

The lumpfish used in this experiment had been reared in Iceland and in the Faroe Islands. The lumpfish were released into the fish pen approximately 2 weeks before sampling began, and the average weight was 30 g. The lumpfish were collected from random Faroese aquaculture pens from Bakkafrost, HiddenFjord and MOWI. The location of the study was in the Northern parts of the Faroe Islands. To keep the companies anonymous, the locations were labelled A, B, C, D and E. Details about the locations can be found in Table 4.1, the significant wave height data (H_{m0}) is taken from Niclasen & Simonsen (2012), and the data about the current was retrieved from the companies that had the aquaculture sites. H_{m0}_50m is the statistically estimated largest 1-hour H_{m0} value during the last 50 years. The current measurements for location D and E are not average current at 10 m dept but measured currents at the localities. Location A, B and C used Faroese lumpfish that were reared in the Faroe Islands and location D and E used Icelandic lumpfish that were reared in Iceland.

Table 4.1. Table containing information about the different localities where lumpfish were sampled.

Location	Start of Sampling	End of Sampling	H_{m0}_50m	Average current at 10m dept
A	16-09-2020	28-10-2020	1-2 m	6 cm/s
B	08-09-2020	04-11-2020	1-2 m	6 cm/s
C	14-09-2020	12-10-2020	2-4 m	5 cm/s
D	15-10-2020	15-10-2020	6 m	50 cm/s (Measured)
E	19-10-2020	19-10-2020	1-2 m	110 cm/s (Measured)

4.2 Sample collection

138 lumpfish were sampled from five different aquaculture sites (Table 4.1) situated in random locations, distributed in the northwest and northeast parts of the Faroe Islands. The sampling was done at the same time as Fiskaaling did their monthly lumpfish examinations, thus monthly samplings. However, location A and B had a different schedule, where Fiskaaling did weekly samplings, and this resulted in a larger sample size from these two locations. All the samplings at the locations were done in the same sea pen.

Figure 4.2. The setup used for measuring density and OWIs. The bucket on the left side contains the live fish, while the bucket on the right contains the sedation and is used to measure the fish weight in water.

The lumpfish were sampled 10 at a time, in a timespan from September to November 2020. The lumpfish that were sampled weighed less than or approximately 200 g. If they weighed more than 200 g, the precision of the weight measurement declined and was 0.1 g instead of 0.05 g. The lumpfish were caught with a dip net (1.5 m pole Ø30 mm, 30x30 net with 5 mm holes) at the edge of the pen. Because lumpfish tend to gulp air when they are exposed to air (observation during the first sampling), it was important to keep them submerged, because air bubbles in the stomach interfere with the weight measurements in water. This was done by keeping the dip net submerged, while the lumpfish were transported from the dip net, into a plastic bag (25x50 cm 6 L) filled with seawater. The lumpfish were then moved from the bag and into a 20 L bucket with seawater.

The lumpfish were then transported alive in the bucket, to the laboratory, which was on the harbour where the aquaculture boats docked. The trip took approximately 10-20 min from the fish pen to the dock. At the laboratory, an aquarium pump was put into the bucket to keep the water oxygenated. The pump used was a PUMP (1.5 W) from Collarglobal (Chernihiv, Ukraine) and is suitable for oxygenating aquariums up to 100 L. When it was time to make the measurements, one lumpfish at a time was moved to another 20 L bucket with seawater, where it was humanely euthanized with an overdose 0.6 g L^{-1} of Finquel (also known as MS-222) (Tjaldurs Apotek, Tórshavn, Faroe Islands).

4.3 Measuring density of lumpfish

The methods for measuring density, used in this study, were modified from Davenport & Kjørsvik (1986). The specific method used was the wire method in the previously mentioned paper. The lumpfish were first weighed in the seawater, this was done by hooking them to a fishhook that was connected to a nylon thread, that was connected to a scale. The scale (Salter 1260 SVDR Precision Electronic Scale (Kent, United Kingdom)) had a measurement accuracy of 0.05 g. After the fish had been weighed in the water, it was weighed in air on the same scale. The temperature and salinity of the seawater was measured for each measurement, so that the density could be calculated (see Equation 4.1).

When the weight of the lumpfish in water and air had been found, the following equations were used to calculate the density of the fish. The equations were modified from Davenport & Kjørsvik (1986).

By using equation 4.1, it was possible to calculate the density of the lumpfish, but to do this some values had to be calculated first. To get all the values needed in equation 4.1; the upthrust from water (u), the volume of the fish (v) and the seawater density (ρ) had to be calculated. This was done in equation 4.2, 4.3 and 4.4, respectively.

Equation 4.1:

$$p = w/v$$

Where:

$p = fish\ density\ (g\ ml^{-1})$

$w =\ weight\ in\ air\ (g)$

$v = volume\ of\ fish$

Equation 4.2:

$$u = w - w'$$

Where:

$w =\ weight\ in\ air\ (g)$

$w' = weight\ in\ water\ (g)$

$u = Upthrust\ from\ water$

Equation 4.3:

$$v = \frac{u}{\rho}$$

Where:

$v = volume\ of\ fish\ (ml)$

$\rho = sea\ water\ density\ (g\ ml^{-1})$

Equation 4.4:

$$\rho(S,T,0) = \rho_0 + A_{SP}S + B_{SP}S^{1.5} + C_{SP}S^2$$

Where:

$S = Salinity\ of\ seawater\ in\ parts\ per\ thousand\ by\ volume\ (ppt)$

$\rho_0 = Density\ of\ pure\ water\ in \frac{kg}{m^3}$

$A_{SP}S + B_{SP}S^{1.5} + C_{SP}S^2 = coefficients\ depending\ on\ the\ water\ temperature$

The values and calculations for these coefficients and ρ_0 are below:

$$\rho_0 = 999.84259$$
$$+6.793952 * 10^{-2} * T$$
$$-9.095290 * 10^{-3} * T^2$$
$$+1.001685 * 10^{-4} * T^3$$
$$-1.120083 * 10^{-6} * T^4$$
$$+6.536332 * 10^{-9} * T^5$$

$$A_{SP} = 0.824493$$
$$-4.0899 * 10^{-3} * T$$
$$+7.6438 * 10^{-5} * T^2$$
$$-8.2467 * 10^{-7} * T^3$$
$$+5.3875 * 10^{-9} * T^4$$

$$B_{SP} = -5.72466 * 10^{-3}$$
$$+1.0227 * 10^{-4} * T$$
$$-1.6546 * 10^{-6} * T^2$$
$$C_{SP} = 4.8314 * 10^{-4}$$

Where:

$T = Temperature\ of\ water$ (℃)

4.4 Calculation of hepatosomatic index

The hepatosomatic index, hereinafter referred to as HSI, is defined as ratio of liver weight to total body weight, and has been used in previous studies regarding lumpfish (Eliasen et al., 2020; Imsland et al., 2020; Willora et al., 2021). An anteroposterior cut was made on the left side of the lumpfish from where the liver was extracted. When the liver had been extracted, the liver weight was measured and the HSI was calculated. The equation for HSI can be found below (Equation 4.5)

Equation 4.5:

$$HSI = 100\frac{Liver\ weight}{Total\ bodyweight}$$

4.5 Calculation of condition factor

To determine the condition factor of the lumpfish Fulton´s K formula (equation 4.6) was used. The formula used in this thesis was modified from Nash et al. (2006)

Fulton´s K is usually calculated from the total length of the fish. However, because we used indicators that affected this length (fin score) we had to use standard length instead. The difference is that total length is measured from the mouth to the end of the tail, while standard length is measured from the mouth to the start of the tail. This makes the Fulton´s K values larger, but the values still tell us about the condition of the fish.

Equation 4.6

$$K = 100\frac{W}{L^3}$$

Where:

$K = The\ condition\ factor$

$W = Weight\ of\ the\ fish$

$L = Standard\ Lenght\ of\ the\ fish\ in\ cm$

4.6 Operational welfare indicators of lumpfish

Looking at the overall health of the lumpfish was important in this study, as the overall health of the fish might affect the lipid deposits of the fish and thus the buoyancy. The overall health of the fish was determined by using the welfare indicators; liver colour (A), fin damage (B) and skin damage (C) as can be seen in Fig. 3.2. The indicators were the same as in Eliasen et al. (2020). More details about these indicators can be found below.

Liver colour 1, 2, 3, 4, 5, and 6 were scored for the fish sampled in the present study. According to Eliasen et al. (2020) liver colour 1 can be an indicator for disease if previous samplings have shown livers of colour 3-4. Liver colour 2-4 are generally considered healthy livers while liver colour 5-6 are considered a sign of starvation.

The skin score used was scored from 1-3, where 1 is good skin with no wounds, 2 is signs of inflammation, while 3 is wounds (Fig. 3.2). The fin score is scaled from 1-3 where 1 is no wear, 2 is some wear and 3 is much wear (Fig. 3.2). The stomach fulness score is a score that goes from 1-3 where 1 is an empty stomach and 3 is a full stomach.

Other welfare indicators were also registered, such as stomach content and what stomach content was most prevalent. If a fish had any signs of disease, it was also noted in the dataset.

4.7 Statistical analysis

All statistical tests were performed in SPSS (USA, Chicago, 233 South Wacker Drive). The dataset itself was first put into Microsoft excel and then the key datapoints were transferred to SPSS. The tests of normality and scatterplots were done in SPSS, while the boxplots were made in Excel. To test for possible differences in response variables a one-way ANOVA was used followed by Tukey multiple post hoc tests in cases of significant ANOVAs (Zar, 1996). An Analysis of Covariance (ANCOVA, Zar, 1996) with density as covariate was used to investigate possible effect of weight and length on fish density.

5 Results

138 lumpfish were sampled in the period between September to November 2020, but 15 samples were not usable making the final sample size 123 fish. When samples were taken from location D (15 in total), the biological developer at the location stated that flavivirus (*Flaviviridae*, CLuV) and kraters disease had been confirmed at the site. The kraters disease was visible on the fish and this was registered in the dataset, but the flavivirus was not possible to reliably confirm without doing PCR. However, a quick examination of the liver pointed towards that most of the fish had flavivirus, which was characterized by a pale and firm liver (Skoge et al., 2018). Because flavivirus has been shown to change the liver colour to pale, these samples were removed from the data analysis.

5.1 Density

The density of the fish sampled, varied from 1.024 g mL^{-1} to 1.036 g mL^{-1} (Fig. 5.1). The density varied between locations (one-way ANOVA, $P < 0.05$, Fig. 5.1), with the lowest density (mean ± SD) found at location E (1.028 ± 0.002 g mL^{-1}), and the highest density found at location B (1.031 ± 0.002 g mL^{-1}).

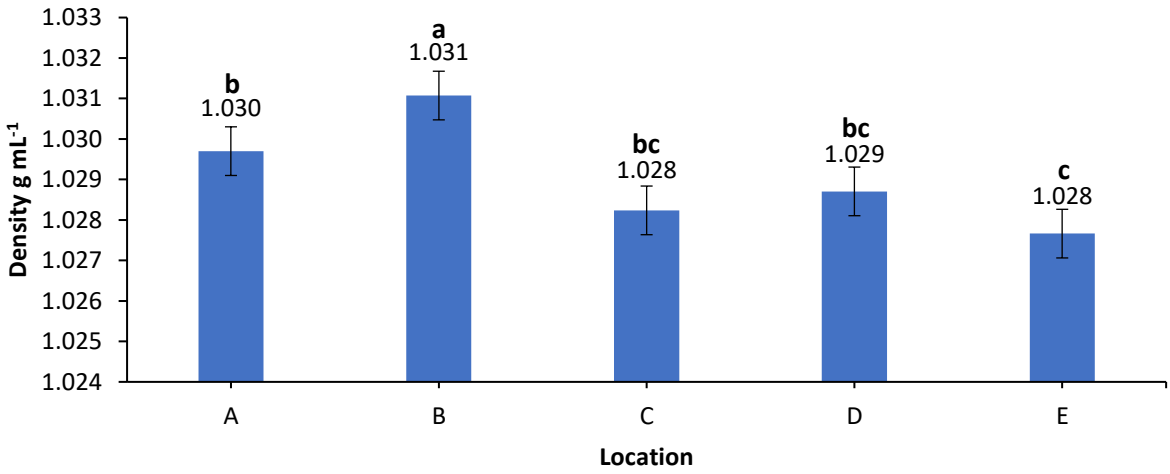

Figure 5.1. Average densities of lumpfish at each location of the study. Different letters indicate significant difference between sampling locations (Tukey´s post hoc test, P<0.05). Further information is given in Appendix A fig. 9-11.

5.2 Fulton´s K.

In Fig. 5.2 the average Fulton´s K values for each location can be seen. No statistical differences in Fulton´s K between sampling locations was found. The average Fulton´s K ± SD value was 5.33 ± 0.80 and the median value was 5.19.

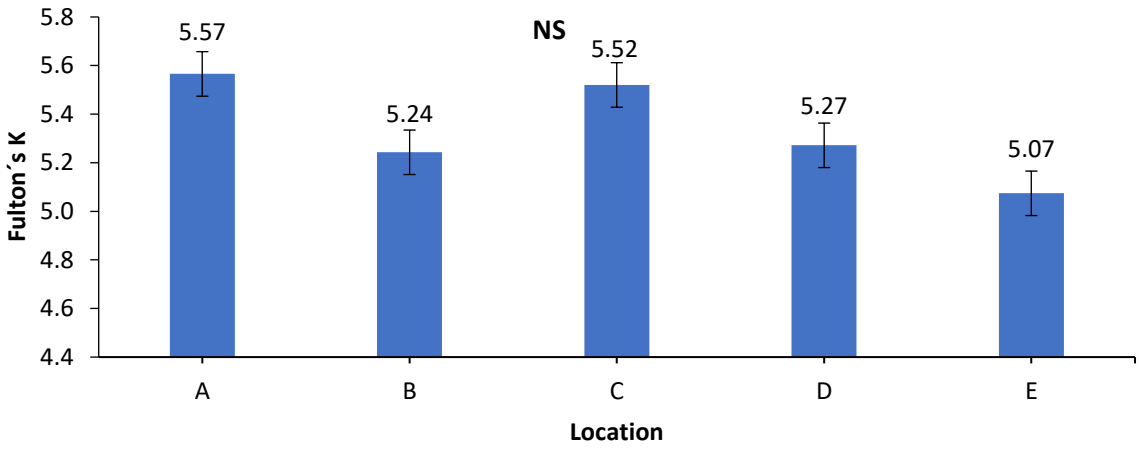

Figure 5.2. Average Fulton´s K values of lumpfish from five different locations in the Faroe Islands. NS indicates no statistical difference between sampling locations (Tukey´s post hoc test, P > 0.05). Further information is given in Appendix A Table. 9.10. Whiskers indicate ± SD.

Fulton´s K was found to negatively influence density (See Figure 5.3) indicating that a higher Fulton´s K value usually had a lower density. This influence was found to be statistically significant (one-way ANOVA, P<0.05,). Because the P-value of the ANOVA test was below 0.05 the H_{01} hypothesis is rejected and the H_{11} hypothesis is accepted indicating that there exists a negative relationship between Fulton´s K and density. However, because the line of fit is so low, these results should be used with caution, as the line of fit could not explain more than 0.8% of the data.

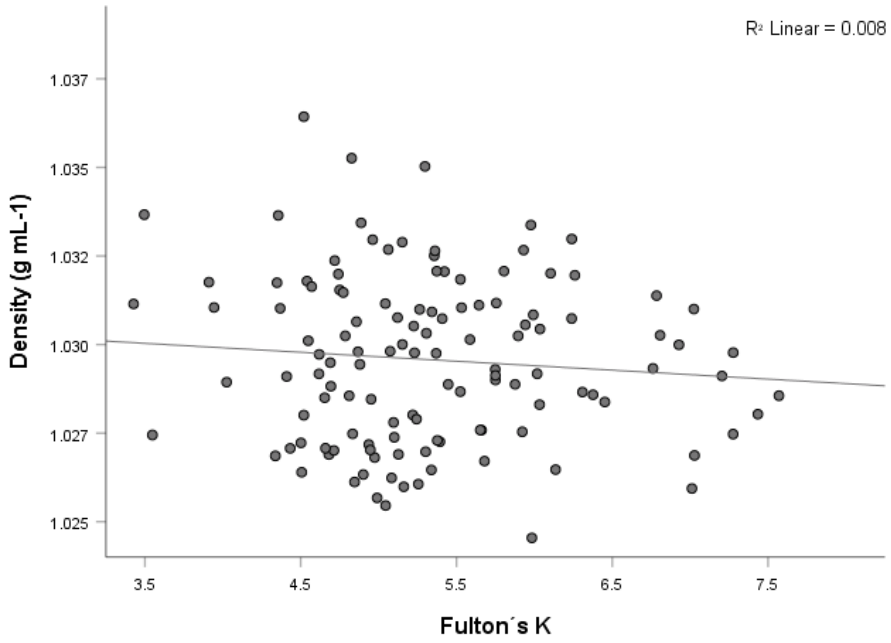

Figure 5.3. A scatterplot with a fit line of density by Fulton´s K.

5.3 Welfare status

Different operational welfare indicators such as fin, skin and stomach scores were compared to density. In Figure 5.4 the density was compared to liver colour and as can be seen in the Figure, the density varied between the liver colours. The liver colour with the highest density was liver colour 3 (1.036 g mL^{-1}), but liver colour 1 had the highest average density (1.031 g mL^{-1}). The liver colour with the lowest density was liver colour 2 (1.025 g mL^{-1}), liver colour 2 also had the lowest average density (1.028 g mL^{-1}). The liver colour with the broadest density range was liver colour 3, spanning from 1.026 to 1.036 g mL^{-1}. The difference in density of the liver colours was statistically significant (one-way ANOVA, $P<0.05$). Tukey´s post hoc test showed that the liver colours were grouped in 3 groups (Fig. 5.4). Liver colours 1, 3 and 4 grouped in one group, liver 3, 4 and 5 in another one, while liver colours 2, 3 and 5 grouped together in the third group.

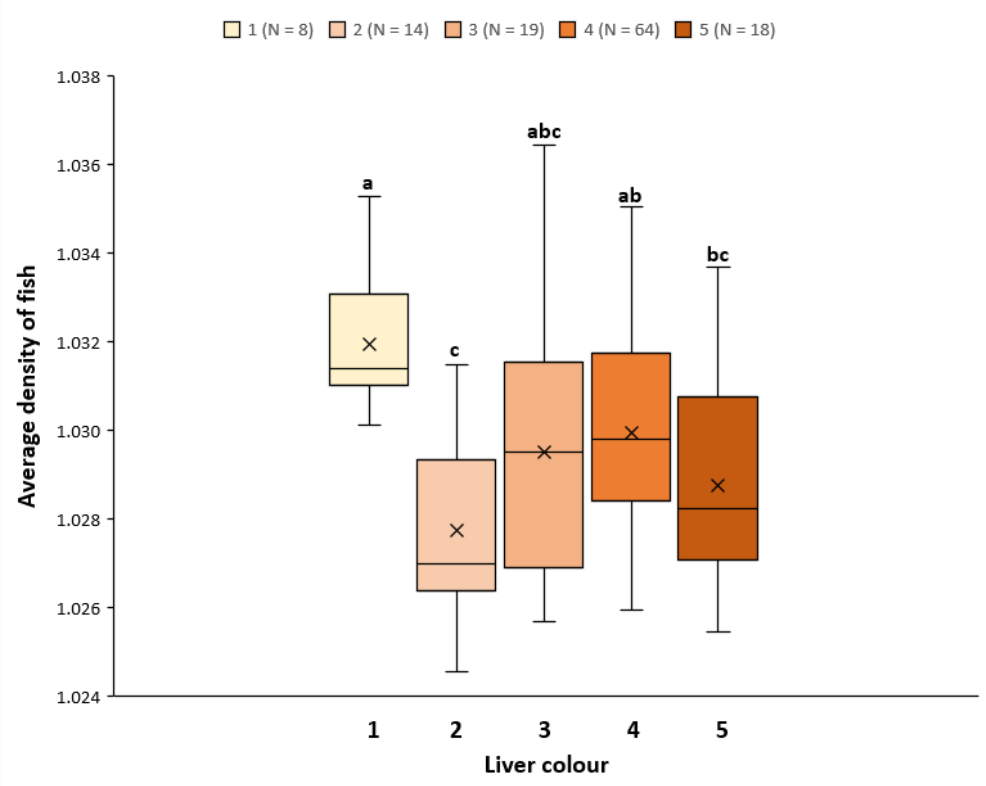

Figure 5.4. Density compared to the liver colour of the lumpfish. Different letters indicate significant differences (Tukey´s post-hoc test, $P < 0.05$) between liver colours. Whiskers indicate minimum and maximum values, while boxes indicate Q1, median, and Q3 quartiles. The X indicates the average density.

When comparing fin and skin score to density (see Table. 9.6 in Appendix A), no statistical significance was found (one-way ANOVA, $P>0.05$). However, the stomach score was statistically significant (one-way ANOVA, $P < 0.05$).

In Table 5.1 averages from different groups (all fish, lowest 30 density fish, highest 30 density fish, and location D) can be seen. The lowest 30 density fish had an average (±SD) density of 1.027 ± 0.007 g mL^{-1} while the highest 30 density lumpfish had a density of 1.033 ± 0.001 g mL^{-1}. The average fin and skin score of the lowest 30 density lumpfish was 1.7 and 1.5 respectively while the highest 30 density lumpfish had an average fin and skin score of 1.6

and 1.2, respectively. The lowest 30 fish had an average stomach score of 2.3 while the highest 30 density fish had an average stomach score of 2.2, which is a difference of 0.1. 21 empty stomachs were found, the prevalence from score 1-3 was the following: 1=21, 2=54, and 3=63. The location with the largest number of empty stomachs was location B which had 15 (32%) empty stomachs.

Table 5.1. The four different groups compared to the different welfare parameters that were used. The values are averages from the measured fish.

Parameters	P-values	All fish (D excluded)	Lowest 30 density fish	Highest 30 density fish	Location D (N = 15)
Density ± SD	NS	1.030 ± 0.002	1.027 ± 0.001	1.033 ± 0.001	1.029 ± 0.002
Fin	0.064	1.52	1.70	1.56	1.73
Skin	0.071	1.32	1.50	1.20	2.07
Stomach	0.006	2.28	2.27	2.17	2.53

5.4 Hepatosomatic index.

The average (±SD) HSI values for each location are showed in Fig. 5.5. Location A and D had the highest (Tukey post hoc test, $P < 0.05$) HSI at 1.51 ± 0.50% and 1.51 ± 0.61%, location B had the third highest HSI at 1.43 ± 0.45%. Location C and E had the lowest HSI at 0.98 ± 0.33% and 0.91 ± 0.37%. The overall average HSI was 1.30 ± 0.51%.

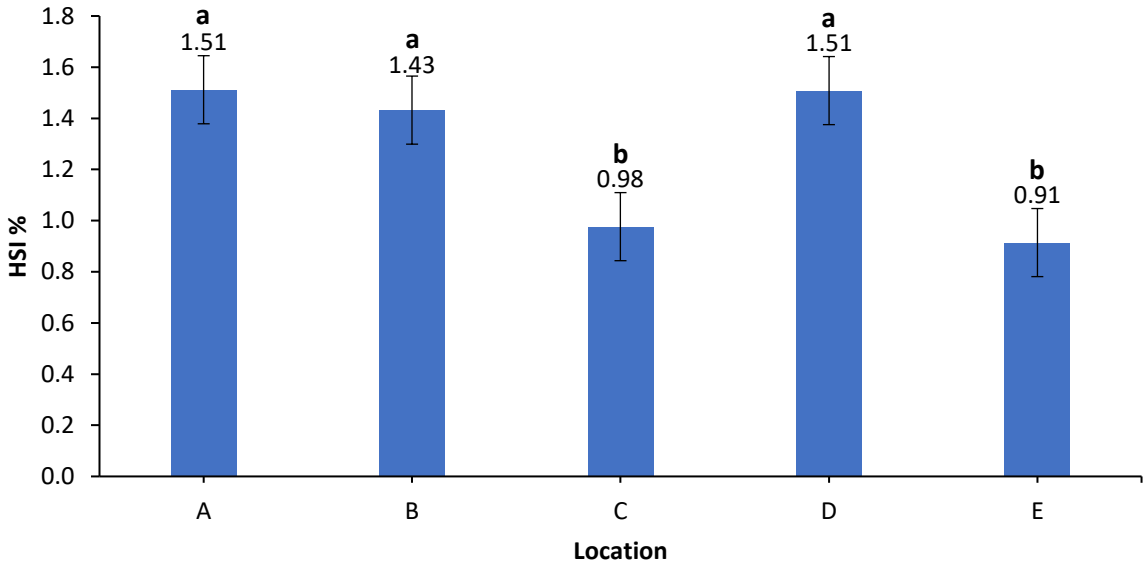

Figure 5.5. The average HSI values for each location. Letters indicate groupings from Tukey´s post hoc test, the test results can be seen in appendix A Table. 9.11. Whiskers indicate ± SD.

HSI was found to positively influence density (See Figure 5.6) indicating that a higher HSI value usually had a higher density. This influence was found to be statistically significant (one-way, ANOVA $P< 0.001$, Fig. 5.6). The line fit was moderate ($R^2 = 0.21$) indicating the fitted line could explain 21% of the data. The slope of the line was 0.03 (see Fig 9.4 in Appendix A) indicating that if HSI increased by 1%, then density increased by 0.03 g mL^{-1}. Because the P-value of the ANOVA test was below 0.05 the H_{03} hypothesis is rejected and the H_{13} hypothesis is accepted, indicating that there exists a positive correlation between HSI and density.

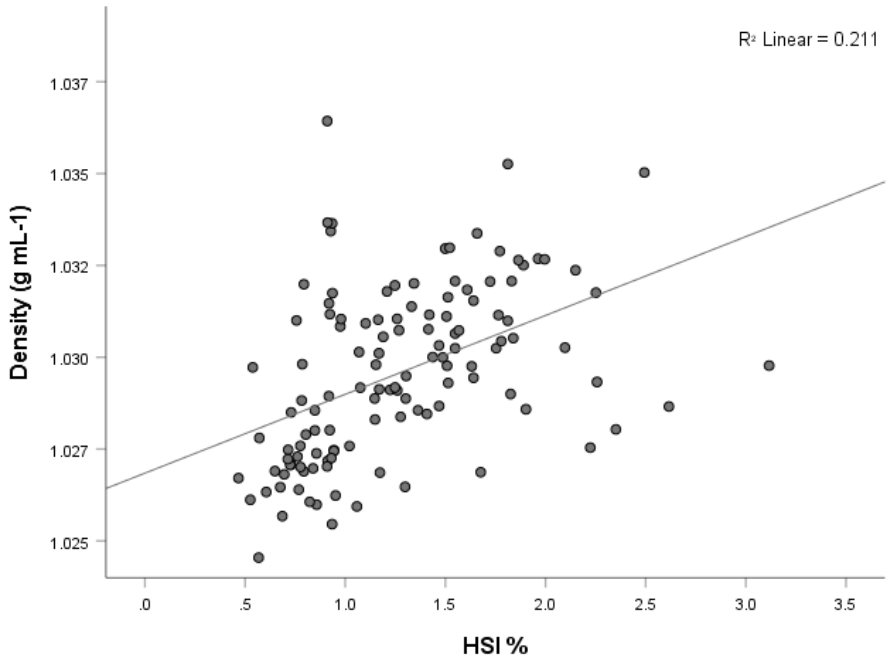

Figure 5.6. A scatterplot with a fit line between lumpfish density by HSI.

5.5 Length and weight

A negative relationship was found between standard length and density (Fig. 5.7). This relationship was statistically significant (two-way ANCOVA, P <0.05), indicating that we should reject the H_{05} hypothesis which states that the standard length of the fish does not influence the density and accept the H_{15} hypothesis which states that the density of the fish is influenced by standard lenght. The slope of the line of fit was -0.01.

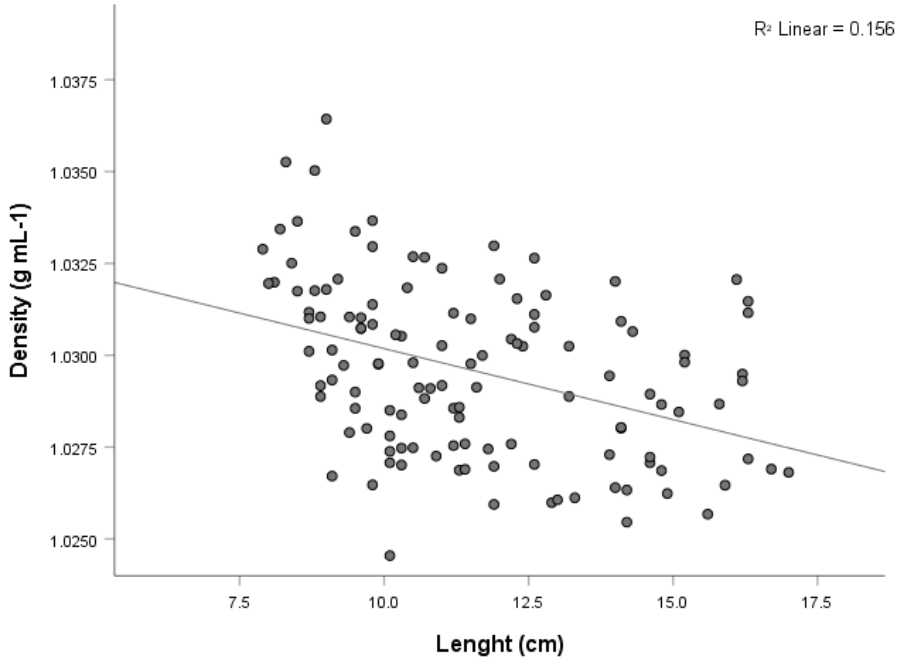

Figure 5.7. A scatterplot with a fit line between lumpfish density and length.

No significant relationship was found between density and weight (Fig. 5.8).

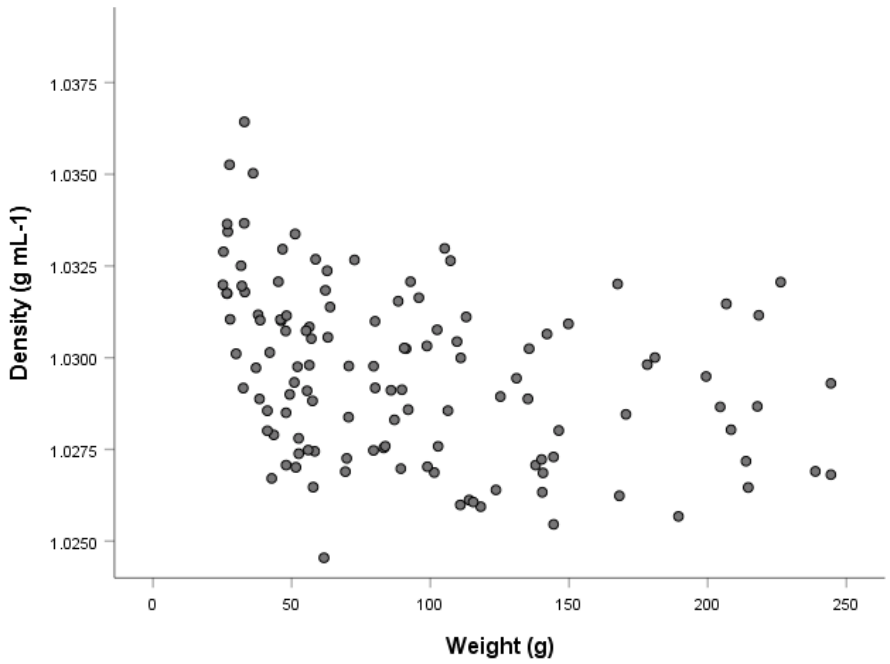

Figure 5.8. A scatterplot between lumpfish density and weight.

6 Discussion

In the present study, density has been measured and shown to be affected by several welfare indicators including Fulton´s K, HSI, liver colour, stomach fullness score, and standard length. Density did not show a statistically significant influence by welfare indicators such as fin score, skin score and weight. However, the P-values of the skin and fin scores tended towards significance (skin score P= 0.071, fin scores P=0.064) indicating that a higher sample size might have resulted in statistical significance.

6.1 Density

The juvenile lumpfish used in this study seem to have a lower density than the adult male lumpfish and a density similar to the female lumpfish used in Davenport & Kjørsvik (1986). However, because the sex of the juvenile lumpfish was not registered in the present study, this will not be discussed further. The similar density to the lumpfish in Davenport & Kjørsvik (1986) indicates that the measurements have been done correctly which strengthens the validity of the dataset used in this study. The similar density to wild adult lumpfish also indicates that the lumpfish used in this study were healthy and in normal condition.

The lumpfish from the different sampling locations used in this study had statistically different densities (Fig. 5.1). The average length of the lumpfish and the sampling time at the locations differed, which might explain the differences. Location A and B were continuously sampled every second week for about two months, while the other locations were sampled on one or two days. Location B differed statistically from all other locations. This might be because it had the smallest average length, as length had a statistically significant relationship with density in present study. The top 30 lumpfish with the highest density, had 21 fish that were from location B and eight from location A, which means only one out of the 30 highest density lumpfish were from another location (Location E). In contrast the lowest 30 density lumpfish were more evenly distributed between locations. The location with the highest number of fish was location E with 16 fish and all the locations had at least one fish represented. The reasons that the locations are different can be many. Firstly, the lumpfish from the locations were from different producers, which might explain the density differences. Imsland et al. (2021) has shown that different lumpfish families have different growth rates, hence it may

be possible that different families also have different densities. It was not possible to get the specific family identification of the fish used in this study, but the country of origin was available and differed between the sampling locations. However, no relationship was found, as location B (which was the only location that had statistically different density) had lumpfish from the Faroe Islands as did location A and C. Secondly the locations themselves might affect the density, as physical forces such as currents or waves might affect the energy usage of the fish. As can be seen in Table 4.1, these two physical factors vary quite a lot between the locations. However, there seems to be no clear relationship between density and these factors, as the density at the locations with higher currents or waves does not differ statistically from the other locations.

Davenport & Kjørsvik (1986) looked at the relationship between density and percentage weight in water compared to in air, to see how the density changes affected the fish. This could not be done in the current study as the salinity of the water in the bucket where the fish was measured had different salinities (21.3 ppt-32.7 ppt). It is possible to do some calculations to offset the salinity change, but this was not done in this study. However, Davenport & kjørsvik (1986) noted that 34 ppt salinity water at a temperature of 5°C had a density of 1.0269 g ml^{-1}, which means that the density of the fish in this study was quite close to the density of saltwater. This indicates that the buoyancy of the fish used in this study was close to neutral.

6.2 Fulton´s K

A negative relationship was found between Fulton´s K and density (one-way ANOVA, $P<0.05$) in the present study. This negative relationship was predicted, as a fish with a high Fulton´s K is fatter and most likely has a larger lipid reserve, which is one of the methods of buoyancy in lumpfish (Davenport & Kjørsvik, 1986). Fulton´s K is used as a condition factor and a high Fulton´s K value is considered good welfare indicator (Imsland et al., 2020), and this is confirmed by the negative relationship to density that found in this study.

All the locations had a high average Fulton´s K value which indicates that the lumpfish used in this study had a good condition. This is also supported by the welfare indicators that were used in this study, as the scores used generally indicated good health (See section 6.3). This is in agreement with Imsland et al. (2020).

The relationship between density and Fulton´s K shows how important it is to keep the lumpfish well fed, as the lumpfish with a low Fulton´s K also have a higher density. This means that a lumpfish with a low Fulton´s K must use more energy to stay afloat. This forces the lumpfish to use up more of their lipid storage which in turn lowers their Fulton´s K and increases their density even more. In the industry this means that if someone is considering starvation intervals as a method of motivating lice eating, it should be done with great caution and by personnel that knows about the relationship between density and Fulton´s K.

6.3 Welfare status

The welfare indicators that showed a statistically significant relationship with density were liver colour, HSI and stomach fullness score. No statistical relationship was found between density and fin/skin score. However, the low scores (where low values are preferable) of the fin and skin indicators, and the high stomach score (High values preferable) indicate that the lumpfish used in this study were healthy (Table 5.1). In addition to this, the large quantity of liver colour 4 also indicate that the lumpfish were healthy (Eliasen et al., 2020), and the low density strengthens this point too.

6.3.1 Comparing the density to liver colours and HSI

The density differences in the liver colours (Fig. 5.4) were mixed evenly between 3 groups: Group 1 (a) contained liver colours 1, 3 and 4, group 2 (b) liver colours 3, 4 and 5, group 3 (c) liver colours 2, 3 and 5. Because the groupings of the liver colour were so evenly distributed and because each group had liver colours spanning wide (group 1 goes from 1-4 etc.) it is difficult to conclude anything from this data and find any biological connections to the groups. According to Eliasen et al. (2020) three different liver colours can be used to identify the overall fish health. A dark liver indicates poor health in terms of low levels of triacyl glycerides, which indicates low lipid reserves (Eliasen et al., 2020). An orange liver indicates an increased level of carotenoid pigments and thus a healthy lumpfish. A pale liver is not necessarily an indicator for bad health, as they are common in smaller lumpfish (>50 g). However, routine monitoring shows that if the distribution of liver colours in an aquaculture sea pen has shifted from orange to bleak it should be taken as a sign of disease (Eliasen et al., 2020). However, according to Davenport & Kjørsvik (1986) the liver does not function as a buoyancy organ for the lumpfish and this is indicated in these results too.

The HSI was measured in the present study (Fig. 6.1 A) to investigate if it was possible to link the density directly to the liver size. Even though the HSI varied, the Tukey's test groupings indicated that liver colours 2, 3 and 5 could be considered one group, while liver colour 4 was another group, and liver colour 1 was between the two groups. Liver colour 4 was shown in Eliasen et al. (2020) to be the liver colour of the healthy lumpfish and to be the liver colour with the highest HSI (Fig. 6.1 B) and this is also the case in present data. However, in Eliasen et al. (2020) liver colour 5 had the lowest HSI which was different from the data in this study.

There are three distinct differences between the data from Eliasen et al. (2020), these being the lack of liver colour 6, the sampling period, and the lower HSI. The lower HSI values can be explained by the accuracy of the weight used. In Eliasen et al. (2020) a spring weight was used that had a measuring accuracy of 0.1 g while the sampling in this study used a kitchen weight with an accuracy of 0.05 g. This measuring difference accounts for the lower HSI values in our dataset, as in Eliasen et al. (2020) it would not be possible to measure down to the same HSI levels.

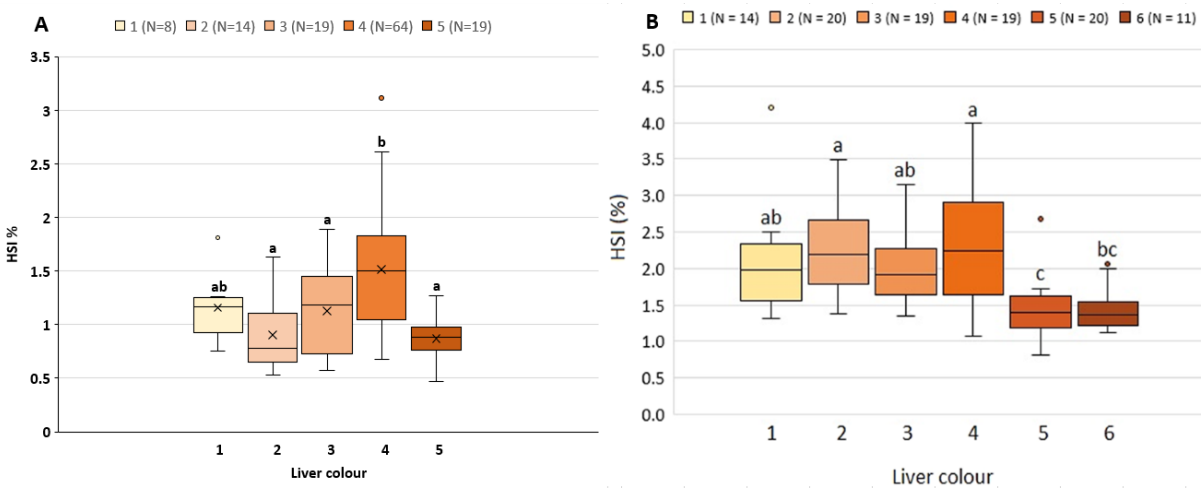

Figure 6.1. Comparison between the HSI for the liver colours from the dataset (Location D excluded) used in this thesis (A) and the HSI of the liver colours from Eliasen et al. (2020) (B)

The sampling period also differed, where Eliasen et al. (2020) sampled all of the fish used for their HSI measurements from the same location at the same day. The present data was sampled at different locations for a period of 2 months. Eliasen et al. (2020) showed that the liver colour distribution varied with size, where the smaller lumpfish (<50 g) had a higher prevalence of dark (5, 6) and light (1, 2) liver colours. This is something that most likely has affected present data, as the fish sampling size varied from 30 g to 200 g. This change in prevalence might explain why no liver colour 6 was found. A rough calculation from the data in Fig. 1 in Eliasen et al. (2020) shows that the prevalence of liver colour 6 in fish under 100 g is approximately 4.5%. This means that between the 138 fish that were sampled, approximately six livers of colour 6 should be found. Because of the low prevalence of liver colour 6 it is quite likely that out of the sampled fish, no fish with liver colour 6 would appear.

Statistical testing indicated that density was influenced by HSI and that density seems to increase as the HSI increases (Fig. 5.6) in line with the results of Davenport & Kjørsvik(1986) are correct. During the sampling, the buoyancy of the livers was tested by dropping the livers into seawater and they did sink, which further indicates that the liver does not contribute to density.

This all supports the existing literature (Davenport & Kjørsvik, 1986) which indicates that the liver does not contribute to buoyancy positively, but rather in a negative way. However, a large liver is most likely still a healthy indicator for a lumpfish as it still indicates higher lipid reserves as mentioned in Eliasen et al. (2020).

6.3.2 Stomach fulness score

Stomach fulness score was the second welfare indicator that had a statistically significant relationship with density in the present study. Linking this score to density would most likely always give statistically significant scores as the stomach content adds to the weight of the fish and thus interferes with the density measurements. However, of the 30 highest density fish, seven had empty stomachs while in the 30 lowest density fish only one had an empty stomach. The average stomach score was not very different between these two groups (Table 5.1).

When comparing the stomach fulness score with liver colours, a pattern emerges. 21 stomachs were empty, and the most prevalent liver colour was liver colour 5 (N=7) followed closely by liver colour 1 (N=6. Liver colours 2 (N=3), 3 (N=2) and 4 (N=3) had a lower prevalence and this is to be expected. A lumpfish with an empty stomach is generally considered a bad indicator of health (Arrington et al., 2002; Eliasen et al., 2018), especially in an aquaculture sea pen where food is in abundance. The fact that the most prevalent liver colour was liver colour 5, which is a sign of hunger (Eliasen et al., 2020), is a good indicator that the stomach fulness score is a useful tool for health indication. However, more sampling would be needed to do statistical tests for this conclusion, as the sample size of 21 fish is too small to compare it to the 5 groups of liver colours. If a link could be established with a stomach score of 1 and a dark liver colour, then stomach score and liver colour could be used as two indicators that confirm each other.

In Eliasen et al. (2020), the darker liver colours had a higher prevalence of empty stomachs than the orange livers. However, the pale livers had a higher prevalence of empty stomachs than liver colour 5, while liver colour 6 had the highest prevalence of empty stomachs. This is similar to present data, with the difference being that the pale livers had a slightly higher

prevalence of empty stomachs. This is most likely because of the low sample size, as the prevalence is difficult to draw conclusions from when only 21 stomachs were empty.

6.3.3 Fin and skin score

The fin and skin score did not have a statistically significant relationship with density in the present study, but the P-value for both scores were close to the P-value threshold of 0.05 (Table 5.1). A fish with a fin score of 3 has no fin at all, and thus it should be expected that this fish needs to spend more energy hunting for food. A fish with a skin score of 3 has wounds and thus its health would most likely be jeopardised and its immune system would be using more energy, it would most likely also have osmoregulatory problems. If these two indicators had a statistically significant relationship to density, then it would most likely be positive. This is because their energy expenditure increases which would most likely make them decrease their lipid reserves which affects the density (Davenport & Kjørsvik, 1986). These operational welfare indicators are commonly used, and in Imsland et al. (2020) it is even recommended that a fish is removed if it has a high score. If density would be affected positively by these two indicators, then it would strengthen the validity of the fin and skin indicators. Thus, further research with a higher sample size is recommended to fully evaluate the fin and skin score and possible relationship with lumpfish density.

6.4 Weight and length

The weight showed no statistical relationship to density, whereas a negative relationship between body length and density was found (Fig. 5.7) i.e., longer fish generally had a lower density. The average standard length for each location was as follows: A=12.9 cm, B= 9.9 cm, C=10.5 cm, D=13.9 cm, and E=14.4 cm. It might be that the larger fish have had time to build up lipid reserves, in their muscles and subcutaneous jelly, and thus have a lower density. This is supported by location B having the lowest average length. Davenport & Kjørsvik (1986), looked at lumpfish larvae and found that they had a significantly lower density, this was most likely caused by a lack of a subcutaneous jelly which had not developed at this life stage. It is possible, that the smaller lumpfish in location B still have not developed their subcutaneous jelly fully and thus have a higher density.

6.5 Flavivirus might affect HSI

An interesting observation that was made during the sampling was the increased HSI of the liver at location D. In Fig. 6.2, a comparison of the dataset with (Boxplot B) and without (Boxplot A) location D is showed. When location D is included, the average HSI for liver colour 1 is above liver colour 4 despite the median still being relatively the same. The increase in HSI is quite remarkable considering that the sample size of liver colour 1 at location D is five. One possible explanation for this is a liver disease caused by flavivirus called *Cyclopterus lumpus* virus (Cluv, Skoge et al., 2018), as location D had confirmed cases of flavivirus which was confirmed with PCR tests. There is a possibility that this was the reason for the higher HSI as flavivirus has been observed to affect the liver of lumpfish (Skoge et al., 2018). Signs of flavivirus include a pale and firm liver (Skoge et al., 2018), but not a higher HSI. It is not possible to link flavivirus to this effect with the data used in this study as no laboratory PCR tests were made to confirm the presence of flavivirus of the samples used in this study. However, this is something that should be investigated further as this is potentially another way of confirming flavivirus cases.

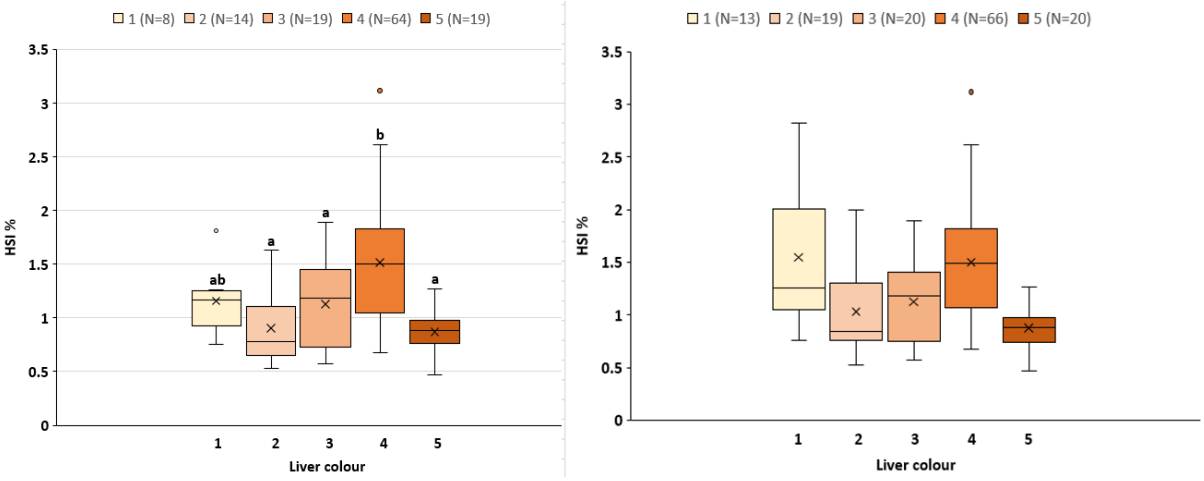

Figure 6.2. Two boxplots showing how the HSI changed from liver colour 1 having a relatively low HSI in boxplot A to having a very high HSI when location D was included in boxplot B. No Tukey´s test was done for boxplot B as the sample sizes were too low.

6.6 Final thoughts/Improvements

The data sampled for this thesis proved to be reliable for its intended use, but there is room for improvements. One of the factors that made the data less reliable was the continuous sampling. A field day where all the samples were taken at once would have improved the result because of the changes that the lumpfish undergo as they grow. Furthermore, a larger sample size (e.g. between 200-300 fish) might have changed some of the results as some of the P-values were close to the threshold of being significant. A more precise weight would also have improved the results, but this might also make the measurements themselves harder. A new version of the scale used in this study has a precision of 0.01 g which would be more suited for these measurements, as the measurements came very close to the scale's capacity. The salinity of the water that the fish was measured in varied quite a lot as the water was taken from the port where the boats docked. This made it harder to do calculations such as percentage weight in water compared to in air, as the low salinity water made the fish weigh more. In future studies it would be better to always use the same salinity as where the fish was sampled.

7 Conclusion

Fulton´s K, liver colour, HSI, standard length, and stomach fulness score were shown to have an influence on density. Fulton´s K stomach score and standard length were shown to have a negative influence while HSI had a positive influence on density.

Density of the juvenile lumpfish used as cleaner fish in Faroese fish farms was shown to be reminiscent to the density of wild female lumpfish. This may suggest that the lumpfish used in this study were healthy, and this was reflected by the welfare indicators measured in this study.

Present findings are relevant for the industry as they have filled an existing knowledge gap concerning density. Additionally, increased knowledge about density and how density is affected by OWIs increases the understanding of the OWIs and makes it easier to understand their effectiveness.

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9 Appendix A

Appendix A contains all the statistical tests that were made for research questions 1-5.

9.1 Descriptive statistics, Normality tests & QQ plots for density, Fulton´s K and HSI

Table 9.1. Descriptive statistics of Density, Fulton´s K and HSI.

Descriptive Statistics

	N	Range	Minimum	Maximum	Mean	Std. Deviation	Variance	Skewness		Kurtosis	
	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic	Std. Error
Fulton_K_STD	123	4.1426	3.4272	7.5698	5.341996	.8377092	.702	.521	.218	.297	.433
Density	123	.0119	1.0245	1.0364	1.029570	.0023452	.000	.277	.218	-.365	.433
HSI	123	2.6489	.4673	3.1161	1.269459	.5018314	.252	.852	.218	.703	.433
Valid N (listwise)	123										

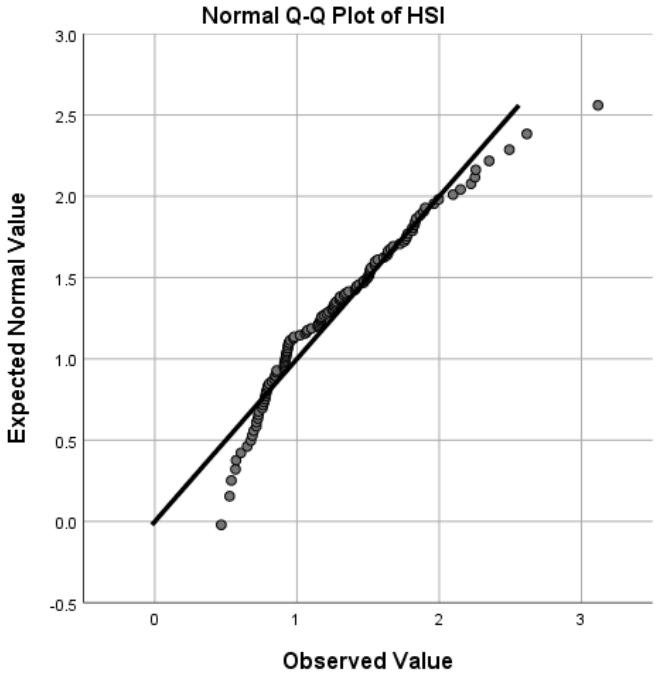

Figure 9.1. A normal QQ plot of HSI the dots aligning reasonably well with the line suggests that the data is normally distributed.

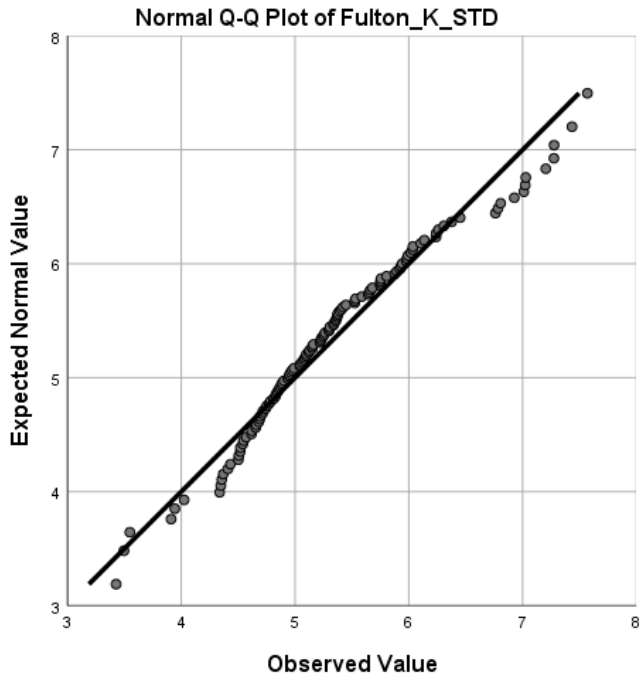

Figure 9.2. A normal QQ plot of Fulton´s K the dots aligning reasonably well with the line suggests that the data is normally distributed.

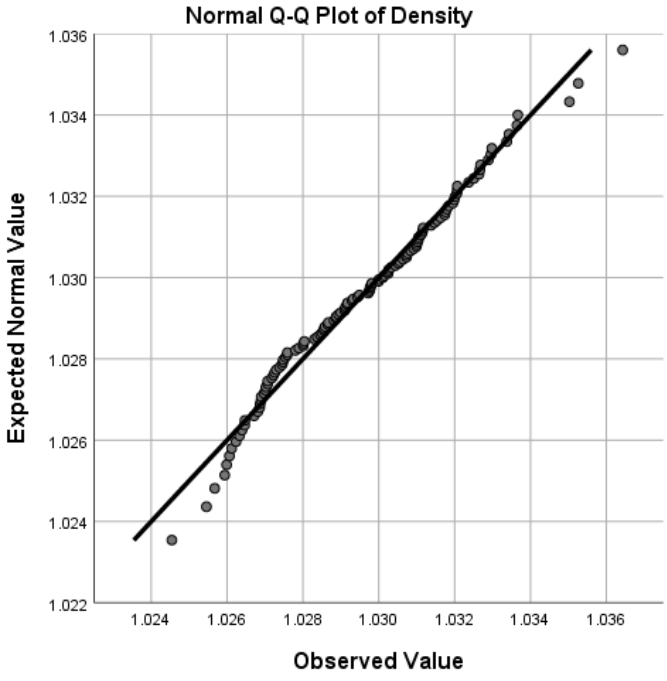

Figure 9.3. A normal QQ plot of Density the dots aligning reasonably well with the line suggests that the data is normally distributed.

9.2 Fulton´s K, HSI and density statistical tests

Table 9.2: ANOVA of HSI and Fulton´s K where both were statistically significant. In the parameter estimates the slope of the lines can be seen.

Tests of Between-Subjects Effects

Dependent Variable: Density

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[b]
Corrected Model	.000[a]	2	9.210E-5	22.704	.000	.275	45.407	1.000
Intercept	3.091	1	3.091	761841.084	.000	1.000	761841.084	1.000
HSI	.000	1	.000	44.061	.000	.269	44.061	1.000
Fulton_K_STD	4.261E-5	1	4.261E-5	10.504	.002	.080	10.504	.895
Error	.000	120	4.057E-6					
Total	130.383	123						
Corrected Total	.001	122						

a. R Squared = .275 (Adjusted R Squared = .262)

b. Computed using alpha = .05

Parameter Estimates

Dependent Variable: Density

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval		Partial Eta Squared	Noncent. Parameter	Observed Power[a]
					Lower Bound	Upper Bound			
Intercept	1.030	.001	872.835	.000	1.028	1.033	1.000	872.835	1.000
HSI	.003	.000	6.638	.000	.002	.003	.269	6.638	1.000
Fulton_K_STD	-.001	.000	-3.241	.002	-.001	.000	.080	3.241	.895

a. Computed using alpha = .05

Table 9.3. Levene´s test of equality of error variances and of heteroskedasticity. Both had a P-Value above 0.05 which is positive for the assumptions of the ANOVA test that was made.

Levene's Test of Equality of Error Variances[a]

Dependent Variable: Density

F	df1	df2	Sig.
1.749	10	112	.078

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + HSI + Fulton_K_STD

Tests for Heteroskedasticity

F Test for Heteroskedasticity[a,b,c]

F	df1	df2	Sig.
.011	1	121	.918

a. Dependent variable: Density

b. Tests the null hypothesis that the variance of the errors does not depend on the values of the independent variables.

c. Predicted values from design: Intercept + HSI + Fulton_K_STD

9.3 Welfare indicators statistical tests

Table 9.4. Results from the statistical test that was done for density compared to liver colours. The P-value was < 0.001 which means that the H_0 hypothesis was rejected.

Tests for Heteroskedasticity

F Test for Heteroskedasticity[a,b,c]

F	df1	df2	Sig.
.108	1	121	.743

a. Dependent variable: Density

b. Tests the null hypothesis that the variance of the errors does not depend on the values of the independent variables.

c. Predicted values from design: Intercept + Liver_Colour

Tests of Between-Subjects Effects

Dependent Variable: Density

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[b]
Corrected Model	.000[a]	4	2.820E-5	5.960	.000	.168	23.841	.982
Intercept	82.751	1	82.751	17492236.50	.000	1.000	17492236.50	1.000
Liver_Colour	.000	4	2.820E-5	5.960	.000	.168	23.841	.982
Error	.001	118	4.731E-6					
Total	130.383	123						
Corrected Total	.001	122						

a. R Squared = .168 (Adjusted R Squared = .140)

b. Computed using alpha = .05

Parameter Estimates

Dependent Variable: Density

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval		Partial Eta Squared	Noncent. Parameter	Observed Power[b]
					Lower Bound	Upper Bound			
Intercept	1.029	.001	2006.670	.000	1.028	1.030	1.000	2006.670	1.000
[Liver_Colour=1]	.003	.001	3.471	.001	.001	.005	.093	3.471	.931
[Liver_Colour=2]	-.001	.001	-1.286	.201	-.003	.001	.014	1.286	.248
[Liver_Colour=3]	.001	.001	1.061	.291	-.001	.002	.009	1.061	.183
[Liver_Colour=4]	.001	.001	2.051	.042	4.105E-5	.002	.034	2.051	.530
[Liver_Colour=5]	0[a]	.	.	.	.	.	.	.	.

Table 9.5. Results from the Tukey´s post hoc test, these results were used for the letter grouping in Fig. 5.4.

Multiple Comparisons

Dependent Variable: Density

	(I) Liver Colour 1,4,6	(J) Liver Colour 1,4,6	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	1	2	.004205*	.0009640	.000	.001535	.006876
		3	.002450	.0009167	.064	-.000090	.004989
		4	.002018	.0008156	.104	-.000241	.004278
		5	.003208*	.0009242	.006	.000648	.005769
	2	1	-.004205*	.0009640	.000	-.006876	-.001535
		3	-.001756	.0007661	.155	-.003878	.000367
		4	-.002187*	.0006417	.008	-.003965	-.000409
		5	-.000997	.0007751	.700	-.003144	.001150
	3	1	-.002450	.0009167	.064	-.004989	.000090
		2	.001756	.0007661	.155	-.000367	.003878
		4	-.000431	.0005682	.942	-.002006	.001143
		5	.000759	.0007154	.826	-.001223	.002741
	4	1	-.002018	.0008156	.104	-.004278	.000241
		2	.002187*	.0006417	.008	.000409	.003965
		3	.000431	.0005682	.942	-.001143	.002006
		5	.001190	.0005803	.249	-.000417	.002798
	5	1	-.003208*	.0009242	.006	-.005769	-.000648
		2	.000997	.0007751	.700	-.001150	.003144
		3	-.000759	.0007154	.826	-.002741	.001223
		4	-.001190	.0005803	.249	-.002798	.000417

Table 9.6. Statistical results for the ANOVA that tested the relationship between different welfare indicators. Stomach score was the only indicator that was statistically significant.

Tests for Heteroskedasticity

F Test for Heteroskedasticity[a,b,c]

F	df1	df2	Sig.
.952	1	121	.331

a. Dependent variable: Density

b. Tests the null hypothesis that the variance of the errors does not depend on the values of the independent variables.

c. Predicted values from design: Intercept + Skin + Fin + Stomach + Skin * Fin + Skin * Stomach + Fin * Stomach + Skin * Fin * Stomach

Tests of Between-Subjects Effects

Dependent Variable: Density

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[b]
Corrected Model	.000[a]	18	7.553E-6	1.468	.117	.203	26.424	.867
Intercept	27.509	1	27.509	5346816.086	.000	1.000	5346816.086	1.000
Skin	2.785E-5	2	1.393E-5	2.707	.071	.049	5.414	.525
Fin	2.907E-5	2	1.453E-5	2.825	.064	.052	5.649	.544
Stomach	5.444E-5	2	2.722E-5	5.291	.006	.092	10.581	.827
Skin * Fin	2.025E-5	3	6.751E-6	1.312	.274	.036	3.936	.341
Skin * Stomach	2.224E-5	3	7.412E-6	1.441	.235	.040	4.322	.372
Fin * Stomach	5.026E-5	4	1.257E-5	2.442	.051	.086	9.769	.682
Skin * Fin * Stomach	1.090E-6	2	5.450E-7	.106	.900	.002	.212	.066
Error	.001	104	5.145E-6					
Total	130.383	123						
Corrected Total	.001	122						

a. R Squared = .203 (Adjusted R Squared = .065)

b. Computed using alpha = .05

9.4 Lenght and weight statistical tests

Table 9.7. F Test for Heteroskedasticity, a p-value above 0.05 is considered good.

Tests for Heteroskedasticity

F Test for Heteroskedasticity[a,b,c]

F	df1	df2	Sig.
.586	1	121	.445

a. Dependent variable: Density

b. Tests the null hypothesis that the variance of the errors does not depend on the values of the independent variables.

c. Predicted values from design: Intercept + Lenght_STD + Weight

Table 9.8. Statistical test between length/weight and density. Because the p-values are above 0.05 the H_0 hypothesis was not rejected.

Tests of Between-Subjects Effects

Dependent Variable: Density

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[b]
Corrected Model	.000[a]	2	5.685E-5	12.241	.000	.169	24.481	.995
Intercept	.862	1	.862	185543.556	.000	.999	185543.556	1.000
Lenght_STD	3.161E-5	1	3.161E-5	6.805	.010	.054	6.805	.735
Weight	8.860E-6	1	8.860E-6	1.908	.170	.016	1.908	.278
Error	.001	120	4.644E-6					
Total	130.383	123						
Corrected Total	.001	122						

a. R Squared = .169 (Adjusted R Squared = .156)

b. Computed using alpha = .05

Parameter Estimates

Dependent Variable: Density

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval		Partial Eta Squared	Noncent. Parameter	Observed Power[a]
					Lower Bound	Upper Bound			
Intercept	1.037	.002	430.748	.000	1.032	1.042	.999	430.748	1.000
Lenght_STD	-.001	.000	-2.609	.010	-.001	.000	.054	2.609	.735
Weight	1.728E-5	1.251E-5	1.381	.170	-7.489E-6	4.204E-5	.016	1.381	.278

a. Computed using alpha = .05

9.5 Tukey´s tests for locations compared to Fulton´s K, HSI, and density

Table 9.9. Tukey´s test results comparing density to the locations where measurements were made.

Multiple Comparisons

Dependent Variable: Density

Tukey HSD

(I) Location	(J) Location	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A	B	-.001374*	.0004499	.023	-.002618	-.000130
	C	.001463	.0005336	.053	-.000013	.002939
	D	.000994	.0006088	.479	-.000690	.002678
	E	.002037*	.0005336	.002	.000562	.003513
B	A	.001374*	.0004499	.023	.000130	.002618
	C	.002837*	.0004899	.000	.001482	.004192
	D	.002368*	.0005709	.001	.000789	.003947
	E	.003411*	.0004899	.000	.002056	.004766
C	A	-.001463	.0005336	.053	-.002939	.000013
	B	-.002837*	.0004899	.000	-.004192	-.001482
	D	-.000469	.0006389	.948	-.002236	.001298
	E	.000575	.0005677	.849	-.000996	.002145
D	A	-.000994	.0006088	.479	-.002678	.000690
	B	-.002368*	.0005709	.001	-.003947	-.000789
	C	.000469	.0006389	.948	-.001298	.002236
	E	.001044	.0006389	.479	-.000724	.002811
E	A	-.002037*	.0005336	.002	-.003513	-.000562
	B	-.003411*	.0004899	.000	-.004766	-.002056
	C	-.000575	.0005677	.849	-.002145	.000996
	D	-.001044	.0006389	.479	-.002811	.000724

Based on observed means.

The error term is Mean Square(Error) = 3.71E-006.

*. The mean difference is significant at the 0.05 level.

Table 9.10. Tukey´s test results comparing Fulton´s K to the locations where measurements were made.

Multiple Comparisons

Dependent Variable: Fulton_K_STD

Tukey HSD

(I) Location	(J) Location	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A	B	.322528	.1869600	.422	-.194521	.839577
	C	.045481	.2217309	1.000	-.567729	.658691
	D	.293902	.2529956	.773	-.405772	.993576
	E	.491512	.2217309	.180	-.121698	1.104722
B	A	-.322528	.1869600	.422	-.839577	.194521
	C	-.277047	.2035865	.654	-.840078	.285983
	D	-.028626	.2372544	1.000	-.684767	.627515
	E	.168984	.2035865	.921	-.394046	.732015
C	A	-.045481	.2217309	1.000	-.658691	.567729
	B	.277047	.2035865	.654	-.285983	.840078
	D	.248421	.2655187	.883	-.485887	.982729
	E	.446031	.2359196	.327	-.206418	1.098481
D	A	-.293902	.2529956	.773	-.993576	.405772
	B	.028626	.2372544	1.000	-.627515	.684767
	C	-.248421	.2655187	.883	-.982729	.485887
	E	.197610	.2655187	.946	-.536697	.931918
E	A	-.491512	.2217309	.180	-1.104722	.121698
	B	-.168984	.2035865	.921	-.732015	.394046
	C	-.446031	.2359196	.327	-1.098481	.206418
	D	-.197610	.2655187	.946	-.931918	.536697

Based on observed means.
The error term is Mean Square(Error) = .640.

Table 9.11. Tukey´s test results comparing HSI to the locations where measurements were made.

Multiple Comparisons

Dependent Variable: HSI

Tukey HSD

(I) Location	(J) Location	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A	B	.079732	.1079271	.947	-.218746	.378211
	C	.535251*	.1279994	.000	.181261	.889240
	D	.003364	.1460476	1.000	-.400539	.407268
	E	.597518*	.1279994	.000	.243528	.951508
B	A	-.079732	.1079271	.947	-.378211	.218746
	C	.455518*	.1175251	.002	.130496	.780541
	D	-.076368	.1369606	.981	-.455141	.302405
	E	.517785*	.1175251	.000	.192763	.842808
C	A	-.535251*	.1279994	.000	-.889240	-.181261
	B	-.455518*	.1175251	.002	-.780541	-.130496
	D	-.531886*	.1532769	.006	-.955783	-.107990
	E	.062267	.1361901	.991	-.314375	.438909
D	A	-.003364	.1460476	1.000	-.407268	.400539
	B	.076368	.1369606	.981	-.302405	.455141
	C	.531886*	.1532769	.006	.107990	.955783
	E	.594153*	.1532769	.002	.170257	1.018050
E	A	-.597518*	.1279994	.000	-.951508	-.243528
	B	-.517785*	.1175251	.000	-.842808	-.192763
	C	-.062267	.1361901	.991	-.438909	.314375
	D	-.594153*	.1532769	.002	-1.018050	-.170257

Based on observed means.

The error term is Mean Square(Error) = .213.

*. The mean difference is significant at the 0.05 level.